Electronic Supplementary Material (ESI) for Lab on a Chip.
This journal is © The Royal Society of Chemistry 2022

Supplementary Information

Filtration-assisted magnetofluidic cartridge platform for HIV RNA detection from blood

Alexander Y. Trick[a], Hoan Thanh Ngo[b], Anju H. Nambiar[a], Marisa M. Morakis[a], Fan-En Chen[a], Liben Chen[b], Kuangwen Hsieh[b], Tza-Huei Wang[a,b,c]

E-mail: thwang@jhu.edu

[a] Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218

[a] Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD 21218

[c] Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218

S1. Blood volume compatibility with filtration module

Experiment was conducted to identify suitable volume of whole blood to be loaded into filtration module. Different volumes of whole blood with the same concentration of HIV particles were prepared by spiking 1uL, 2uL, 4uL of HIV particles (10^3 $TCID_{50}$/mL) into 5uL, 10uL, 20uL of whole blood respectively. The mixtures were vortex vigorously and loaded onto filtration module. PBS (200uL) was injected into filtration module. Plasma was collected using 1.5mL Eppendorf LoBind tubes with dried MES binding buffer (see below). The tubes were vigorously vortexed and incubated for 1min. After that, the tubes were places on a Magnet rack DynaMag™-2 Magnet (ThermoFisher). Zymo beads were pulled out of solution, and supernatants were removed by pipetting. The beads were washed once with 50 uL of 20% PEG w/v in 0.5% Tween-20. Zymo elution buffer (10uL) was added onto the beads and 1uL of eluate was added to 6.5uL PCR master mix in PCR tube. PCR amplification was conducted using a benchtop realtime PCR system BioRad CFX96 (5 min 50 ^oC RT, 10 sec 95 ^oC hotstart, 50 cycles of 1 sec 95 ^oC and 1 sec 60 ^oC). Ct values were recorded and compared with a standard curve of different concentration of HIV particles directly spiked into PCR master mix to calculate HIV recovered and % recovery.

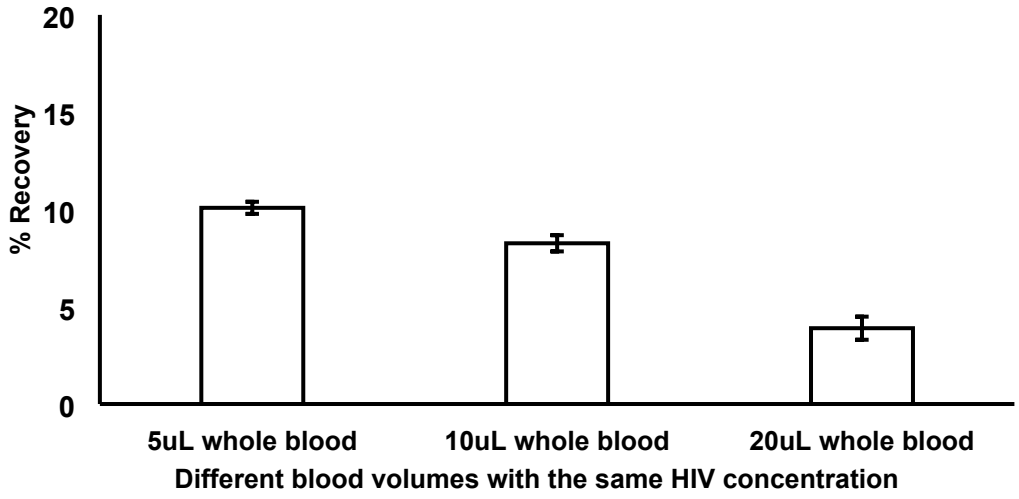


Figure S1. HIV viral particle recovery with varying blood volumes after filtration and bead capture

S2. Filter membrane evaluation

10 µL whole blood spiked with 1 µL 10^4 $TCID_{50}$/mL HIV particles was filtered through 3 different candidate membranes (PCTE = Sterlitech 2 µm polycarbonate track-etched filters, Vivid = Pall Vivid plasma separation membrane, CA = Sterlitech 5 µm cellulose acetate filters) using 100 µL 1X phosphate buffered saline (PBS). Unfiltered blood mixed with PBS, PBS alone with spiked viral particles, and the resulting filtrates were each mixed with 1 µL 10% v/v Tween-20, 4 µL Chargeswitch magnetic beads, and 10 µL Chargeswitch binding buffer to lyse and capture viral RNA. Beads were pelleted and washed with 50 µL Chargeswitch W12 wash buffer followed by pelleting and elution into 10 µL Chargeswitch elution buffer. RNA recovery was calculated with qPCR containing 1 µL of each eluate with each condition run in duplicate. PCR reactions were 10 µL in total composed of 1X lyoready PCR mix (Meridian Biosciences), 0.125 µL PrimeScript reverse transcriptase enzyme mix (Takara), 0.3 µM primers, 0.2 µM probe, and 1 µL eluate as target. PCR was run on a Biorad CFX96 thermocycler using 10 min. 42°C reverse transcription hold followed by 40 cycles of 95°C denaturation for 5 seconds and 60°C annealing and extension for 20 seconds. Recovery percentage was calculated using quantification by cycle threshold (Ct) using a standard curve generated by direct spike of synthetic HIV RNA (Fig. S2A). Overall recovery is calculated based on Ct comparison to direct spike of HIV particles into PCR solution (Fig. S2B).

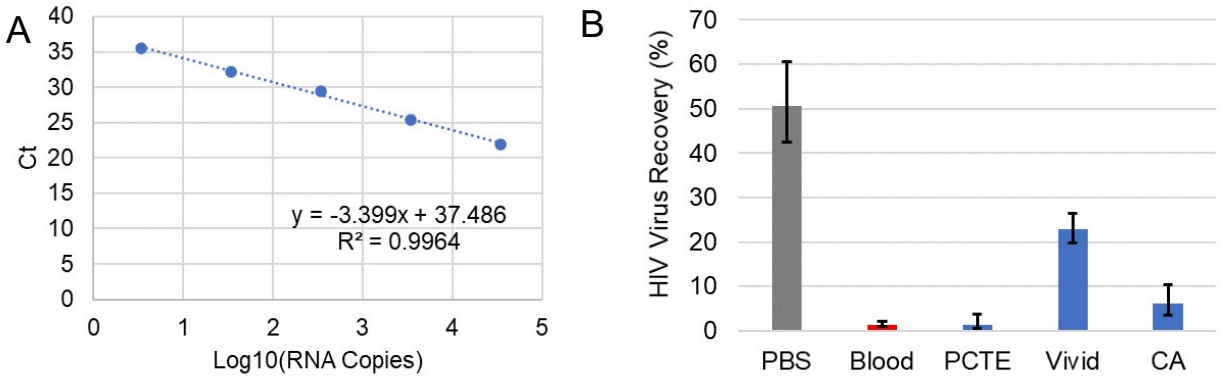
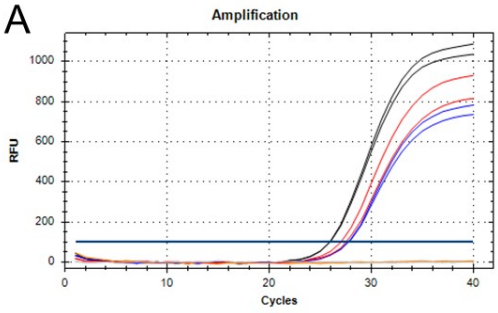


Figure S2. Filter membrane with bead capture viral recovery. A – Standard curve generated with synthetic HIV RNA copies. B – Recovery percentage using Chargeswitch beads of 10^4 $TCID_{50}$/mL HIV particles spiked into 100 µL PBS without filtration (PBS), 100 µL PBS with 10 µL blood without filtration (Blood), or 100 µL PBS with 10 µL blood after filtration through polycarbonate track-etched filters (PCTE), Pall Vivid plasma separation membranes (Vivid), or cellulose acetate filters (CA).

S3. Chargeswitch vs. Silica bead interaction with blood

To compare the inhibitory effect of carryover from blood on Chargeswitch versus Zymo Magbinding magnetic beads, 4 µL Chargeswitch beads or Zymo beads were mixed with 20 µL whole blood and 20 µL of either Chargeswitch binding buffer or Zymo M-Bind buffer, respectively. Beads were pelleted onto a magnetic rack and Chargeswitch beads were washed twice with 200 µL Chargeswitch W12 wash buffer, while Zymo beads were washed twice with 200 µL either 80% v/v isopropanol (IPA) in water, Zymo M-Wash, or Chargeswitch W12 buffer. Wash buffers were removed and replaced with 20 µL PCR buffer using the same recipe described in S2 spiked with 20,000 copies of synthetic HIV RNA (ATCC). After bead exposure, the PCR was split into two 10 µL reactions and run on a Biorad CFX96 thermocycler as described in S2. For comparison, two 10 µL PCR reactions pre-spiked with 10^4 copies synthetic HIV RNA each were run with no exposure to beads. Zymo beads washed with Chargeswitch buffer showed slightly earlier Ct values compared to Chargeswitch beads, indicating less inhibition (Fig. S3A). All Zymo beads washed in organic solvents resulted in complete inhibition of PCR. During extraction from blood, Zymo beads were easily extracted from the blood solution, while Chargeswitch beads remained primarily trapped in the blood volume (Fig. S3B). The loss of Chargeswitch beads aspirated with the blood prior to wash steps may have reduced the overall inhibitory effect that would have resulted from full carryover of the beads into PCR .

A



Test Condition	Rep 1 - Ct	Rep 2 - Ct
No Beads	25.9	25.94
Chargeswitch Beads	27.75	27.69
Zymo Silica Beads + 80% IPA Wash	N/A	N/A
Zymo Silica Beads + M-Wash (ethanol)	N/A	N/A
Zymo Silica Beads + Chargeswitch Wash	27.02	27.49
No Template Control	N/A	N/A

B

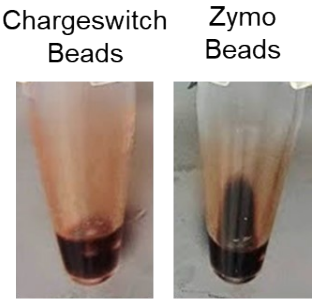


Figure S3. Chargeswitch vs. Zymo silica bead assay inhibition. A – Real-time PCR fluorescence curves for reaction exposed to beads after binding in blood and washing with a variety of buffers indicated by color. Ct values for each replicate (Rep) of each condition are listed as reported by the Biorad CFX software. B – Chargeswitch beads in blood against a magnetic rack showed severely reduced attraction to the side walls, while silica beads were easily attracted out of the blood.

S4. PEG wash titration

In addition to PEG, salts are typically added to solutions to facilitate nucleic acid precipitation and retention during wash steps. To assess the effect of salt concentration on nucleic acid recovery, 1 µL 10^3 $TCID_{50}$/mL HIV particles spiked into 50 µL water were bound onto 3 µL Zymo Magbinding beads with 45 µL 5M GuSCN 100mM MES binding buffer and 1 µL Triton-X 100. Beads were washed with 50 µL of either water, 1X PBS, 20% PEG w/v in 1X PBS, 20% PEG w/v in water, or 20% PEG w/v in water supplemented with 10 mM, 50 mM, or 100 mM of either potassium chloride (KCl) or magnesium chloride (MgCl). After washing, beads were eluted into 10 µL Zymo elution buffer. 1 µL of each eluate was spiked into 9 µL PCR buffer for a final concentration of 1X Quantabio qScript XLT one-Step RT-qPCR TouchMix, 0.5 µM primers, 0.25 µM probe, 1 mg/mL bovine serum albumin (BSA, New England Biosciences), and 0.1% v/v Tween-20. PCR was run on a Biorad CFX96 thermocycler using 10 min. 50°C reverse transcription hold followed by a 30 second hotstart at 95°C and 50 cycles of 95°C denaturation for 5 seconds and 60°C annealing and extension for 20 seconds.

Out of all conditions, 20% PEG w/v in water without any salt supplemented produced the highest recovery of RNA as indicated by the lowest Ct (Fig. S4). Both salt solutions produced the most recovery with 50 mM KCl or MgCl, though the added salt in these PEG solutions or with PBS may have resulted in downstream PCR inhibition causing the higher Ct values.

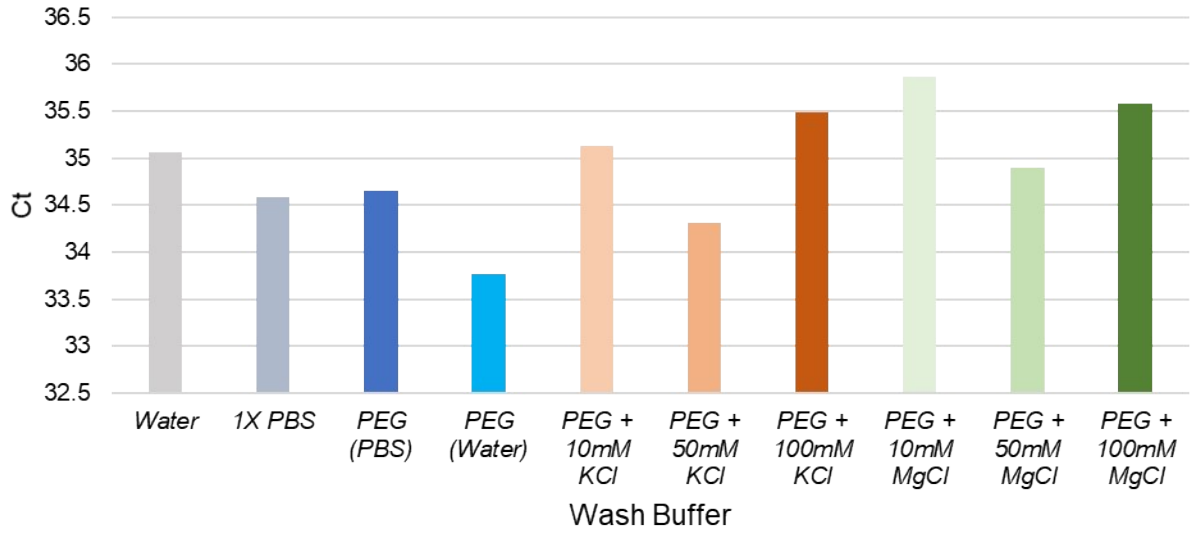


Figure S4. PEG wash salt titration

S5. Ethanol versus PEG wash

To evaluate performance of our aqueous PEG wash buffer compared to more standard 70% ethanol wash, 3 µL Zymo Magbinding beads were first bound with 1 µL 10^3 $TCID_{50}$/mL HIV viral particles spiked into 50 µL 1X PBS mixed 150 µL binding buffer (4M GuSCN in 55 mM Tris HCl pH 7.5 with 25 mM EDTA and 3% v/v Triton-X-100). 6 tubes of this bead mixture were pelleted with a magnetic rack and washed with 50 µL 70% ethanol, while beads in another 6 tubes were pelleted and washed with 50 µL 20% w/v PEG. Half of the 70% ethanol tubes and half of the PEG tubes had their solutions covered with 100 µL silicone oil (100 cSt, Sigma-Aldrich) for each step with aspiration of only the aqueous buffers between steps. To simulate cartridge conditions in the oil covered tubes, beads were manipulated purely by magnetic attraction, while tubes without oil were subjected to thorough resuspension via vortexing during each step. After washing, the beads were eluted into 10 µL Zymo elution buffer and 1 µL of each eluate was spiked into 6.5 µL PCR buffer. The final composition of the PCR buffer was 1X TaqMan Lyo-ready 1-Step RT-qPCR master mix, 0.5 µM HIV primers, 0.25 µM HIV probe, and 1 mg/mL BSA. Rapid PCR conditions were run with a Biorad CFX96 with a 5 minute reverse-transcription hold at 50°C followed by a 10 second hotstart at 95°C with 50 cycles of 1 second denaturation and annealing holds at 95°C and 60°C respectively.

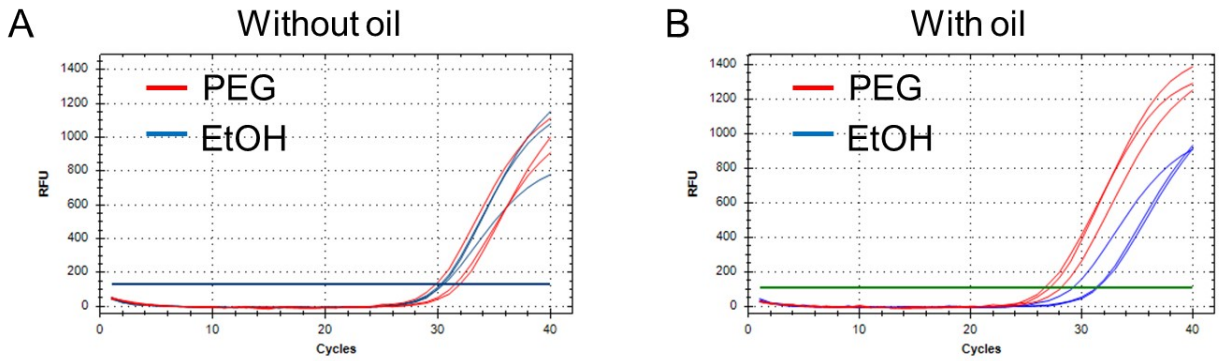


Figure S5. Ethanol versus PEG wash. Real-time amplification of eluted HIV RNA after wash with 20% w/v PEG or 70% ethanol (EtOH) without oil (A) or with silicone oil covering reagents throughout (B).

S6. Binding buffer comparison

Four different binding buffers were prepared. MES binding buffer aliquots were prepared by mixing 150uL of a stock solution (5M GuSCN in 0.1M MES), 1uL 100% Triton X-100, and 3uL Zymo silica magnetic beads inside 1.5mL Eppendorf LoBind tubes. The solutions were then dried by keeping the tubes in a vacuum chamber overnight with lids open. The tubes (containing dried MES binding buffers) were kept in a desiccator cabinet until use. Magnesium acetate (MgOAc) binding buffer aliquots were prepared by mixing 150uL of a stock solution (5M GuSCN in 0.1M MgOAC) and 2uL 100% Triton X-100. Tris-HCl binding buffer aliquots were 150uL of 4M GuSCN, 55 mM Tris HCl pH 7.5, 25 mM EDTA, and 3% Triton X-100. Tris-HCl + isopropanol (IPA) binding buffer aliquots were prepared by adding 150uL IPA to 150uL Tris-HCl binding buffer.

To compare performance of the binding buffers, 150uL of binding buffers and 3uL of Zymo silica magnetic beads were added to 150uL of plasma in 1.5mL Eppendorf LoBind tubes. To get 150uL of plasma: 1uL of HIV particles with TCID50 1E3/mL was spiked into 10uL of whole blood; the whole blood spiked with HIV particles was loaded onto filter membrane inside blood filtration module; 200uL of phosphate buffer saline (PBS) was injected onto the membrane by a syringe; a 150uL of plasma came out and was collected in a clean 1.5mL Eppendorf LoBind tube. 150uL of binding buffer and 3uL of Zymo silica magnetic beads were added to the collected plasma. An exception is for the dried MES binding buffer. Eppendorf LoBind tube that was used to collect plasma was tube containing dried MES binding buffer (see above). And mixture of plasma, binding buffer, and Zymo beads were obtained by vigorous vortexing so that plasma could reconstitute the dried MES binding buffer without any further addition of binding buffer or Zymo beads.

Mixtures of plasma, binding buffer, and Zymo beads were incubated for 10 mins under shaking so that Zymo beads could capture HIV RNA released into the mixtures. After that, the tubes were placed on a Magnet rack DynaMag™-2 Magnet (ThermoFisher). Zymo beads were pulled out of solution, and supernatants were removed by pipetting. The beads were washed once with 50uL of 20% PEG in 0.5% Tween. An exception is for Tris-HCl + IPA buffer. The beads were washed once by 50uL of 50% ethanol (EtOH) and once with 50uL of RNase-free water.

After bead washing, 6.5uL of PCR master mix and 1.0uL of RNase-free water were added onto the washed beads to elute HIV RNA bound on surface of Zymo beads. Eluant were transferred into PCR tubes, and realtime PCR was conducted on a benchtop realtime PCR system BioRad CFX96 (5 min 50 ^{o}C RT, 10 sec 95 ^{o}C hotstart, 50 cycles of 1 sec 95 ^{o}C and 1 sec 60 ^{o}C). Ct values were recorded to compare performance of different binding buffers.

S7. HIV viral particle to copy number standard curve and conversion

PCR as described in S5 was formulated and spiked with 10-fold serial dilutions of synthetic HIV RNA from 10^4 to 10 copies per reaction or 1 µL HIV viral particles from 10^3 to 10 $TCID_{50}$/mL and run on a Biorad CFX96 as described in S5. The resulting standard curve with synthetic RNA was used to calculate the relationship between $TCID_{50}$/mL and synthetic genome copy number to be 1 $TCID_{50}$/mL = 19.9 copies/µL (standard deviation = 9.8 copies).

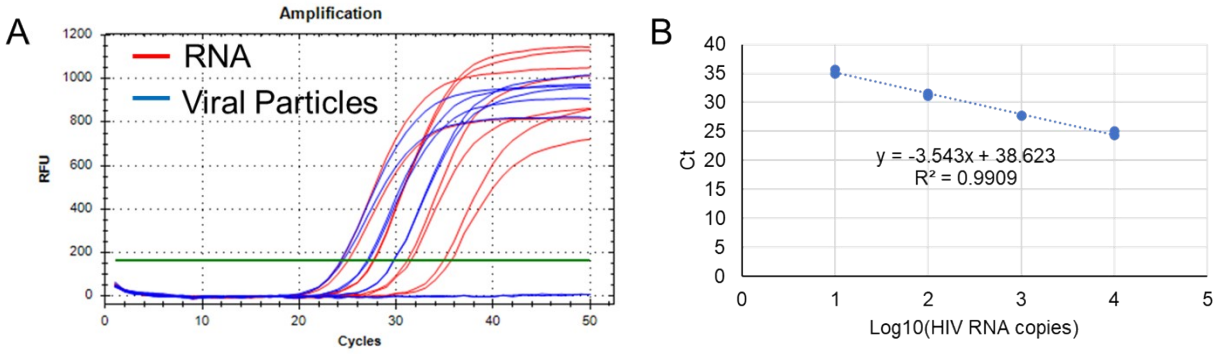


Figure S6. Standard curve and viral load to synthetic RNA conversion. A – Real-time PCR fluorescence curves for serial dilutions of synthetic HIV RNA versus viral particles. B – Standard curve generated by Ct values of synthetic RNA serial dilutions.

S8. Direct spike on cartridge

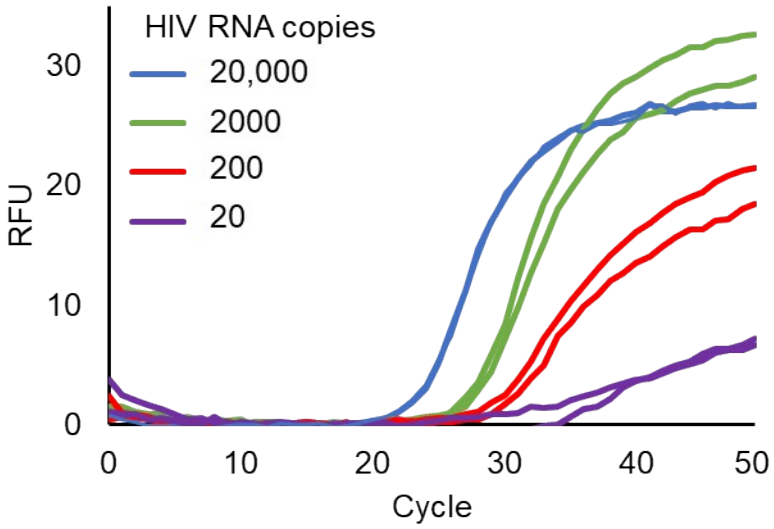


Figure S7. PCR fluorescence curves for cartridges with HIV particles spiked directly into PCR (n = 2).

S9. RNA extraction from larger plasma volumes

HIV viral particles (1 µL containing either 100 or 1000 copies) was spiked into 100 µL aliquots of human blood plasma obtained from whole blood by centrifugation at 2000g for 10 minutes. For each aliquot, RNA was bound to magnetic beads and washed in 1.5 mL microcentrifuge tubes using either the MagMAX Blood RNA isolation kit protocol and reagents or the binding and wash reagents used for cartridges (n=3 for each condition except n=2 for 1000 copies processed by MagMax). Beads were suspended in reagents with manual pipetting with the aid of a magnetic rack for pelleting beads to allow supernatant extraction to exchange solutions. After the wash steps, beads were each directly eluted into 7.5 µL of PCR solution and PCR eluate was amplified as previously described on the Biorad CFX96 system. All 1000 and 100 copy samples amplified using the MagMAX protocol, indicating a limit of detection at 1000 copies/mL of plasma or lower (Fig. S8). For the custom reagent protocol, only the 1000 copy samples amplified. These results indicate with the current formulations and a filtration module capable of separating 100 µL of plasma, the limit of detection could be improved to 10,000 copies/mL. If bead chemistry is optimized to match RNA recovery of the MagMAX kit, then limit of detection could be further improved to 1000 copies/mL or better.

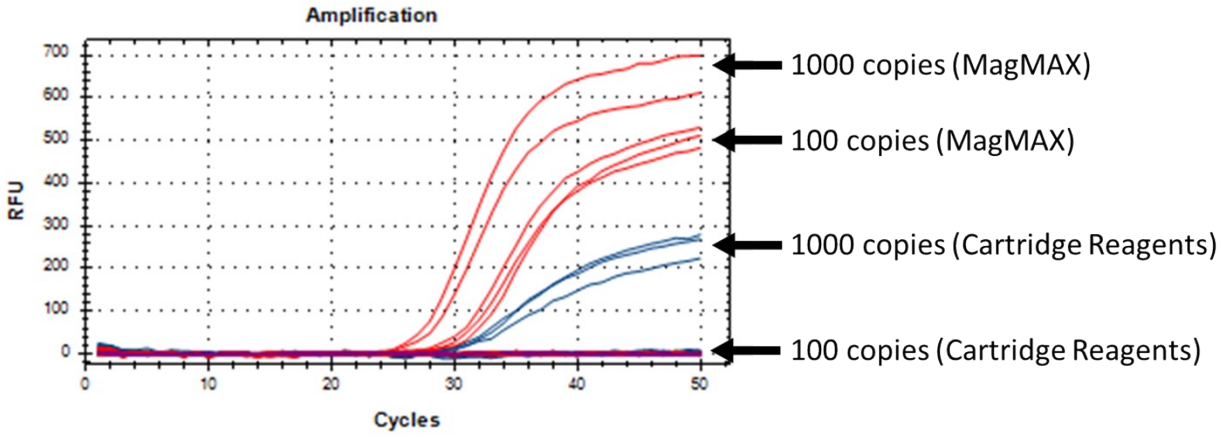


Figure S8. Real-time PCR curves from direct elution of magnetic beads. HIV RNA was extracted from 100 µL plasma using custom cartridge reagents or the MagMax Blood RNA Isolation kit protocol and reagents.